

## Experimental Investigation on the Pathogenesis of Tartrazine-Induced Asthma

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ISHIHARA, Y. and KITAMURA, S. *Experimental Investigation on the Pathogenesis of Tartrazine-Induced Asthma.* Tohoku J. exp. Med., 1979, 129 (3), 303-309 — The effects of tartrazine on the synthesis of prostaglandin-like substances (PGLS) from arachidonic acid in isolated perfused guinea pig lung, and on the contractile responses of guinea pig tracheal tissues induced by histamine, acetylcholine, bradykinin, serotonin and prostaglandin  $F_{2\alpha}$  were studied. The synthesis of PGLS from arachidonic acid was not inhibited by tartrazine. The contractile responses of guinea pig tracheal tissues induced by various bronchoconstrictors were potentiated in the presence of tartrazine. These results may suggest that tartrazine-induced asthma is not induced by inhibition of PGLS synthesis, but induced by potentiation of bronchoconstrictor responses. — tartrazine, PGLS, arachidonic acid; asthma; guinea pig lung lobes; guinea pig tracheal tissues

Tartrazine (FDC Yellow No. 5) is widely used as a color additive for foods and medications. Recently, it has been reported in this and foreign countries that bronchial asthma is induced by foods and drugs containing this color additive (Freedman 1977). The tartrazine induced asthma is regarded as a sort of drug-induced asthma. Aspirin-induced asthma is well known as the representative among drug-induced asthma.

In previous experiments on pathophysiology of aspirin-induced asthma using isolated perfused guinea pig tracheal tissues, we demonstrated that relaxation of tracheal tissues induced by various bronchodilators was markedly suppressed with continuous infusion of aspirin (Ishihara et al. 1978) and that contraction of tracheal tissues induced by various bronchoconstrictors was markedly potentiated with continuous infusion of aspirin (Ishihara et al. 1977).

The present investigation was conducted to demonstrate the effect of tartrazine on biosynthesis of prostaglandin-like substances (PGLS) from arachidonic acid (AA) in the isolated perfused guinea pig lung, and on contractile responses of guinea pig tracheal tissues induced by various bronchoconstrictors.

### MATERIALS AND METHODS

#### *Detection of PGLS synthesized from arachidonic acid in the isolated perfused guinea pig lung*

Male Hartley strain guinea pig, weighing 250-300 g, were killed. Heart and lungs were removed en masse and the apical part of the heart was cut away. A polyethylene

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catheter was inserted through left ventricle into left auricle and tied carefully at the mitral valve area. This lung and heart preparation was then suspended in a bioassay glass funnel and perfused through the catheter with Krebs-Henseleit solution containing 25% fluosol-43 at 37°C saturated with oxygen and carbon dioxide (95:5, v/v). The rate of perfusion flow was kept constant at 10 ml/min. Drugs were infused into pulmonary vein or onto bioassay tissues with a low-speed infusion pump (Harvard Apparatus, USA). For the detection of PGLS, the effluent from the right ventricle was superfused over an isolated rat colon. The contractile responses of the rat colon were detected by an isotonic transducer (ME Commercial, Japan) and displayed on a polyrecorder (Fig. 1A).

The grade of contraction is expressed as the contraction index (C.I.) measuring the area enclosed by response curve and the base line using a planimeter. The inhibition rate of PGLS synthesis from AA in the isolated perfused lung is expressed as a per cent inhibition of PGLS synthesis calculated as follows.

$$\text{Per cent inhibition of PGLS synthesis} = \left( 1 - \frac{\text{C.I. (l) drug} - \text{C.I. (d) drug}}{\text{C.I. (l) cont} - \text{C.I. (d) cont}} \right) \times 100$$

where C.I. (l) drug, C.I. of AA in the isolated perfused lung with continuous infusion of tartrazine or indomethacin; C.I. (l) cont, C.I. of AA in the isolated perfused lung without

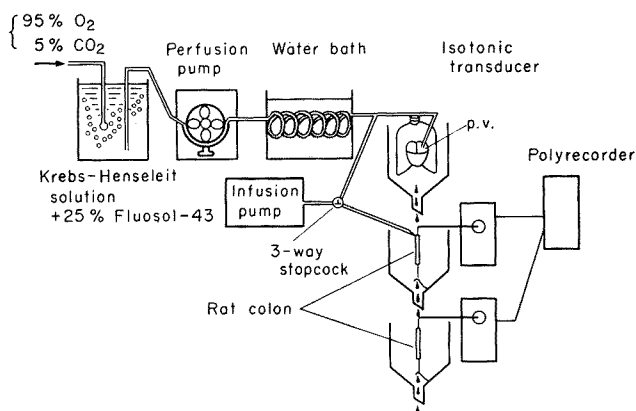


Fig. 1A

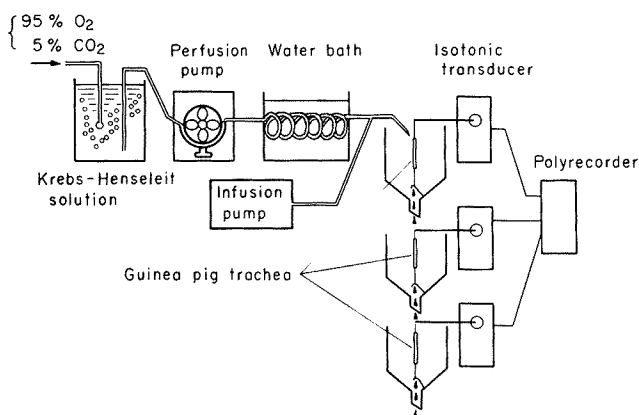


Fig. 1B

Fig. 1. Tissue bioassay system.

infusion of tartrazine or indomethacin (control); C.I. (d) drug, C.I. of AA infused directly on assay tissues with continuous infusion of tartrazine or indomethacin; C.I. (d) cont, C.I. of AA infused directly on assay tissues without infusion of tartrazine or indomethacin (control).

#### Tissue bioassay system

Male Hartley strain guinea pigs, weighing 250–300 g, were killed, and the trachea was removed, cut spirally 1.0–1.5 mm in width and 3–4 cm in length and suspended in a bioassay glass funnel.

Fig. 1 (B) illustrates the schema of tissue bioassay system. Guinea pig tracheal tissues were superfused with Krebs-Henseleit solution at 37°C saturated with oxygen and carbon dioxide (95:5, v/v). The superfusion flow rate was constant at 20 ml/min. Various drugs except tartrazine were infused with a low-speed infusion pump (Harvard Apparatus, USA) at the rate of 0.97 ml/min for 60 sec. Tartrazine was infused continuously in the same way, and usually 10 min after the beginning of its continuous infusion various bronchoconstrictors were infused for 60 sec. Contraction of tracheal tissues was detected by an isotonic transducer (ME Commercial, Japan) and displayed on a polyrecorder.

Drugs used were tartrazine (Yellow No 5, Sanei Chemical, Japan), arachidonic acid (AA, Sigma Chemical, USA), indomethacin (Merck, USA), flusol-43 (Midori-juji, Japan), acetylcholine (Ach, Daiichi Pharmaceut, Japan), histamine dihydrochloride (Takaracosan, Japan), bradykinin (Protein Research Foundation, Japan), serotonin creatinine sulfate (5HT, Daiichi Chemical, Japan), prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>, Ono Pharmaceut., Japan).

Fig. 2 shows the structural formula of tartrazine.

The significance of differences in experimental data obtained here was analyzed by the unpaired *t*-test.

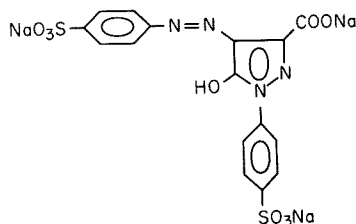


Fig. 2. The structural formula of tartrazine.

## RESULTS

Fig. 3 shows per cent inhibition of PGLS synthesis by tartrazine and indomethacin in the isolated perfused guinea pig lung. Mean values and standard deviation (s.d.) of per cent inhibition of PGLS at the dose of 0.5, 5, 50, and 500 ng/ml of tartrazine were  $0.05 \pm 0.09\%$ ,  $0.07 \pm 0.11\%$ ,  $0.07 \pm 0.08\%$ , and  $0.13 \pm 0.14\%$ , respectively, while those at the dose of 500 ng/ml of indomethacin were  $62.7 \pm 3.5\%$ .

There was no significant difference between per cent inhibition of PGLS by different doses of tartrazine, while there was a significant difference between per cent inhibition of 500 ng/ml of tartrazine and that of 500 ng/ml of indomethacin ( $p < 0.001$ ;  $n = 10$ ).

Fig. 4 shows the effect of different doses of tartrazine on the contractile responses of guinea pig tracheal tissues induced by histamine ( $2.5 \times 10^{-7}$  g/ml). Histamine-induced contractile responses of guinea pig tracheal tissues were

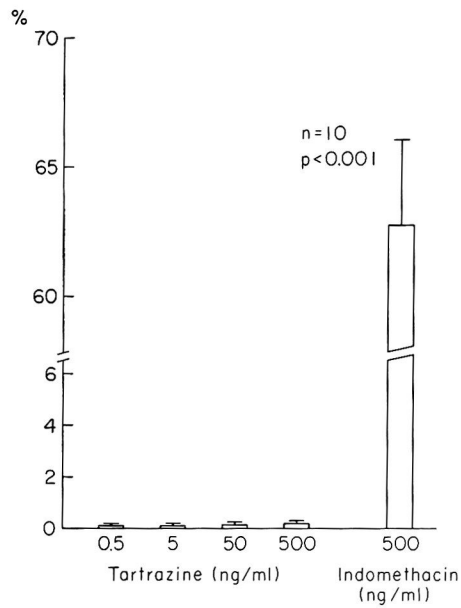


Fig. 3. Per cent inhibition of PGLS synthesis by tartrazine and indomethacin.

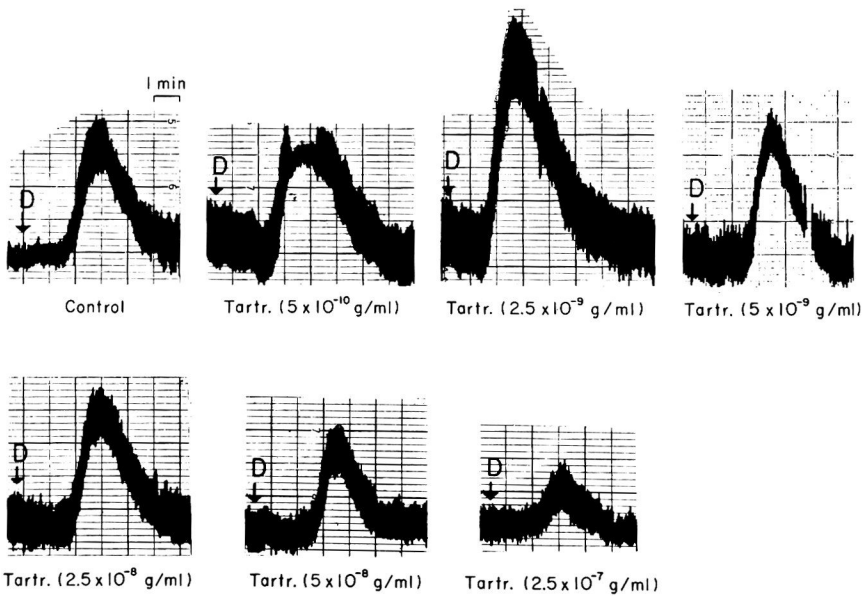


Fig. 4. Effect of different doses of tartrate on the contractile responses of guinea pig tracheal tissues induced by histamine ( $2.5 \times 10^{-7}$  g/ml).

potentiated in the presence of tartrazine, and such a potentiation reached the maximum at the dose of  $2.5 \times 10^{-9}$  g/ml of tartrazine.

Fig. 5 summarizes the above experimental data (solid circles) together with the results on contractions induced by Ach ( $5 \times 10^{-7}$  g/ml) (open circles) and

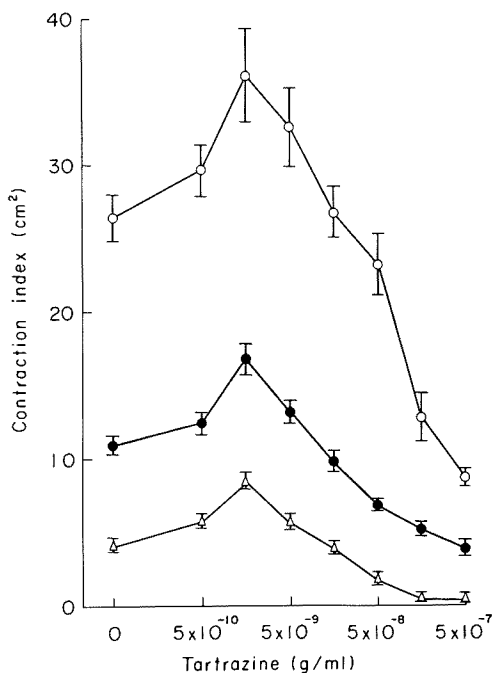


Fig. 5. Effect of different doses of tartrazine on the contractile responses of guinea pig tracheal tissues induced by acetylcholine ( $5 \times 10^{-7}$  g/ml), histamine ( $2.5 \times 10^{-7}$  g/ml) and bradykinin ( $5 \times 10^{-8}$  g/ml).

TABLE 1. Effect of different doses of tartrazine on the contractile responses of guinea pig tracheal tissues induced by acetylcholine, histamine, serotonin and prostaglandin  $F_{2\alpha}$

Concentration of tartrazine (g/ml)	Contraction index (cm <sup>2</sup> )				
	Acetylcholine ( $5 \times 10^8$ g/ml) n=8	Histamine ( $2.5 \times 10^{-7}$ g/ml) n=10	Bradykinin ( $5 \times 10^{-8}$ g/ml) n=8	Serotonin ( $2.5 \times 10^{-7}$ g/ml) n=8	Prostaglandin $F_{2\alpha}$ ( $2.5 \times 10^{-7}$ g/ml) n=8
0	26.43 ± 1.49	10.85 ± 0.49	4.31 ± 0.29	12.09 ± 0.53	5.08 ± 0.30
$5 \times 10^{-10}$	29.59 ± 1.72†	12.40 ± 0.72*	5.93 ± 0.44*	15.49 ± 0.86*	6.00 ± 0.29*
$2.5 \times 10^{-9}$	36.23 ± 3.07*	16.89 ± 0.90*	8.55 ± 0.47*	23.98 ± 1.39*	6.28 ± 0.32*
$5 \times 10^{-9}$	32.64 ± 2.62*	13.32 ± 0.73*	5.84 ± 0.48†	24.84 ± 0.94*	8.24 ± 0.43*
$2.5 \times 10^{-8}$	26.88 ± 1.70 <sup>N.S.</sup>	9.86 ± 0.66†	3.98 ± 0.33*	26.26 ± 1.11*	6.96 ± 0.52*
$5 \times 10^{-8}$	23.34 ± 2.07†	6.82 ± 0.30*	1.94 ± 0.28*	19.65 ± 1.08*	3.88 ± 0.32*
$2.5 \times 10^{-7}$	12.78 ± 1.61*	5.15 ± 0.44*	0.60 ± 0.13*	18.93 ± 0.96*	3.16 ± 0.31*
$5 \times 10^{-7}$	8.66 ± 0.58*	3.92 ± 0.48*	0.48 ± 0.13*	18.65 ± 0.86*	1.78 ± 0.28*

Statistical significance was determined by t-test between each value and its control.

\*  $p < 0.001$ ; †  $p < 0.01$  ‡  $p < 0.05$ ; N.S., non-significant.

bradykinin ( $5 \times 10^{-8}$  g/ml) (open triangles). The detailed figures for mean  $\pm$  s.d. are given in Table 1. C.I.'s of Ach, bradykinin and histamine were increased in the presence of tartrazine with the maximum at the dose of  $2.5 \times 10^{-9}$  g/ml.

In the same way Fig. 6 shows the effect of different doses of tartrazine on the contractile responses of guinea pig tracheal tissues induced by 5HT ( $2.5 \times 10^{-7}$  g/ml) and  $\text{PGF}_{2\alpha}$  ( $2.5 \times 10^{-7}$  g/ml). The mean values and s.d. of C.I. are given also in Table 1. C.I.'s of 5HT and  $\text{PGF}_{2\alpha}$  were increased in the presence of tartrazine, reaching the maximum at the dose of  $2.5 \times 10^{-8}$  g/ml of tartrazine in the case of 5HT and at the dose of  $5 \times 10^{-9}$  g/ml of tartrazine in the case of  $\text{PGF}_{2\alpha}$ .

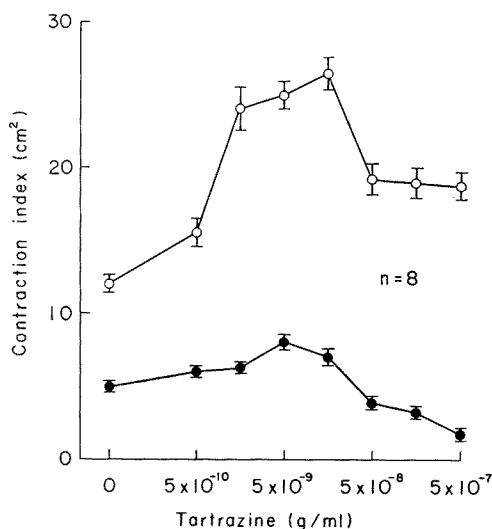


Fig. 6. Effect of different doses of tartrazine on the contractile responses of guinea pig tracheal tissues induced by serotonin ( $2.5 \times 10^{-7}$  g/ml) and prostaglandin  $\text{F}_{2\alpha}$  ( $2.5 \times 10^{-7}$  g/ml).

## DISCUSSION

Tartrazine (0.5–500 ng/ml) did not inhibit synthesis of PGLS from AA in the isolated and perfused guinea pig lung. Ach-, bradykinin-, histamine- and  $\text{PGF}_{2\alpha}$ -induced contractile responses of guinea pig tracheal tissues were potentiated in the presence of tartrazine and they reached the maximum at the dose of  $2.5 \times 10^{-9}$  g/ml. With further increase in its dose, the contractile responses decreased. 5HT-induced contractile responses of guinea pig tracheal tissues were also potentiated in the presence of tartrazine, reaching the maximum at the dose of  $2.5 \times 10^{-8}$  g/ml of tartrazine. But these potentiating and attenuating effects completely disappeared within 20 min after withdrawal of tartrazine. These results may suggest that the potentiating effect of tartrazine on the actions of various bronchoconstrictors is not mediated by inhibition of PGLS synthesis. The potentiating mechanism is not elucidated yet, but it is conceivable that the potentiation might be resulted

from the change of cell membrane permeability to  $\text{Ca}^{++}$  in guinea pig tracheal smooth muscle cells or from the change of intracellular cyclic nucleotide contents in guinea pig tracheal smooth muscle. On the other hand, it is conceivable that the attenuation at the extremely higher dose of tartrazine might be resulted from the adverse change of cell membrane permeability to  $\text{Ca}^{++}$  or adverse change of intracellular cyclic nucleotide contents in guinea pig tracheal smooth muscle.

Tartrazine has a widespread use in various medications by itself or combined with other color additives. This color additive has been used not only for coloring tablets and capsules but also for dosage identifying agent, and it has been used also as a color additive for foods and cosmetics.

According to the report from Food Protection Committee in National Research Council held at Washington D.C. in 1971 (National Research Council 1971), it has been estimated that the maximum ingested dose of tartrazine is 16.3 mg per day.

In 1959, Lockey (1959) reported three patients who developed allergic type reactions after ingesting dexamethasone or prednisolone colored by tartrazine, and in addition he reported that one of these patients showed hypersensitivity to acetylsalicylic acid.

The clinical symptoms and signs of tartrazine-induced asthma are not so drastic that patients by themselves may often miss the causative agents which induce asthmatic attacks. So, physicians should always be alert to pay attention to such conditions.

#### References

- 1) Freedman, B.J. (1977) Asthma induced by sulphur dioxide, benzoate and tartrazine contained in orange drinks. *Clin. Allergy*, **7**, 407-415.
- 2) Ishihara, Y., Sasaki, K., Kitamura, S., Kosaka, K. & Harasawa, M. (1977) Experimental investigation on the pathogenesis of aspirin-induced asthma. The effect of aspirin on the contraction of guinea pig tracheal tissues with various bronchoconstrictors. *Jap. J. thorac. Dis.*, **15**, 380-384. (Japanese)
- 3) Ishihara, Y., Yotsumoto, H., Sasaki, K., Kudoh, S. & Kitamura, S. (1978) Experimental investigation on the pathogenesis of aspirin-induced asthma. The effect of aspirin on the relaxation of guinea pig tracheal tissues with various bronchodilators. *Jap. J. thorac. Dis.*, **16**, 202-206. (Japanese)
- 4) Locky, S.D. (1959) Allergic reactions due to FD & Yellow No. 5, tartrazine, an aniline dye used as a coloring and identifying agent in various steroids. *Ann. Allergy.*, **17**, 719-721.
- 5) National Research Council (1971) Food Protection Committee, Food colors, Washington, D.C., National Academy of Sciences.